Applied Microscopy
Korean Society of Microscopy

pISSN 2287-5123 · eISSN 2287-4445
http://dx.doi.org/10.9729/AM.2015.45.4.254

Regular Article

The Structure of Visual Cells in the Retina of the Pond Loach, *Misgurnus anguillicaudatus* (Pisces; Cobitidae)

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Received September 14, 2015
Revised October 14, 2015
Accepted October 20, 2015

A histological study on the retina of *Misgurnus anguillicaudatus* was carried out by light microscopy and scanning electron microscopy as part of getting information about relation between its habitat and visual cells. The visual cells of the retina in *M. anguillicaudatus*, a bottom-dwelling freshwater pond loach in stagnant or slow waters such as swamps, reservoirs and paddy fields, consists of double cones and large rods. The cones form a row mosaic pattern in which the partners of double cones are linearly oriented with a large rod. In a double cone, the two members are unequal such that one cone may be longer than the other.

Key Words: *Misgurnus anguillicaudatus*, Retina, Rods, Double cones, Row mosaic

INTRODUCTION

The visual cell layer of the teleost eye contains 2 types of photoreceptors, rods and cones, which process visual information at different levels of ambient illumination. The rod cells detect only the presence or absence of light which is resulting in dim light, while the cone cells are responsible for bright light and color vision. (Hagedorn et al., 1998; George & Robert, 2001; Kim & Park, 2002). In most teleosts, the rod cells are much more slender than the cone cells and difficult to find in the retina, whereas the cone cells, containing single and double cones with a regular arrangement known as the cone mosaic pattern, are commonly observed and arranged into regular, heterotypic mosaics containing one, two or three cone cells (Lyall, 1956; Rossetto et al., 1992; Yuko et al., 1997). Teleost are known to have a duplex retina consisting of both rod cells and cone cells. But in a few shallow-water species cones are sparse or even absent (e.g., cusk-eels) and most deep-sea teleost have usually pure rod retinae (Nicol, 1989). In particular, the variations of the cone cells, including their densities and patterns, may be related to aspects of species ecology such as feeding habits and photic habitats, as well as environmental conditions (Lyall, 1957; Collins & MacNichol, 1978; Fernald, 1988; Frank et al., 2001; Thomas & Craig, 2010; Kim et al., 2014).

Pond loach, *Misgurnus anguillicaudatus*, is a freshwater fish belonging to the loach family Cobitidae. They inhabit shallow, slow-moving sections of rivers and streams or calm habitats such as swamps, reservoirs and paddy fields, with substrates composed of mud or slit. With small heads and small eyes, these benthic fish are diurnal and mainly feed on algae and aquatic insects (Kim & Park, 2002). Little is known about the visual cells in this species.

In this study, therefore, we describe the morphology and arrangement of the visual cells, focusing on the relationship between morphology, environment and habitat.

MATERIALS AND METHODS

Ten specimens were collected from Jeonmi-dong, Deokjin-

This research was supported by a grant from the National Fisheries Research and Development Institute (R2015037).

gu, Jeonju-si, Jeollabuk-do on the Mankyeong River in Korea (35°53´N; 127°06´E) during the non-spawning season. The eyes were extracted after being anaesthetized with MS-222 (200 mg/L^{-1}) and fixed in 10% neutral buffered formalin. Fragments were dehydrated through a standard ethanol series to 100%, cleared in xylene, and embedded in wax (Paraplast; Leica, Germany). Five-micrometer sections were deparaffinized and then stained with Harris hematoxylin and counter-stained with eosin (Gurr, 1956) for general histology. For photographs and evaluation of the eyes, Carl Zeiss Vision was used (LE REL 4.4; Carl Zeiss, Germany). For scanning electron microscopy (SEM), the fragments were prefixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2. Postfixation was performed in 1.0% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series and drying to a critical point with liquid CO_2, the dried samples were coated with gold by ion sputtering and then examined with a SEM (S-450; Hitachi, Japan). For transmission electron microscopy (TEM), using the same method in fixation and dehydration as SEM, the fragments were embedded in epon mixture (Epon 812; EMS, USA). They were then observed with a TEM (H7650; Hitachi). Serial semithin sections (0.5~1.0 μm thick) were stained with toluidine blue and examined with the light microscope (LM) for gross morphology. Both radial and tangential sections were examined at right angles and parallel to the plane of the retina, respectively.

RESULTS

External Morphology of Eye

Based on external morphology, this species has small eyes on a small head: the horizontal eye diameter is 2.24±0.34 mm and 15.29%±2.13% to standard length. The eyes are transparent and are very similar in size; each is elliptical with the horizontal dimension being long in comparison to the short perpendicular length (Fig. 1A). There are no apparent eyelids.

General Structure of the Retina

In a radial section by light microscope, the retina contains several layers. Layers from the outermost layer to the layer closest to the vitreous body include a choroid layer, a retinal

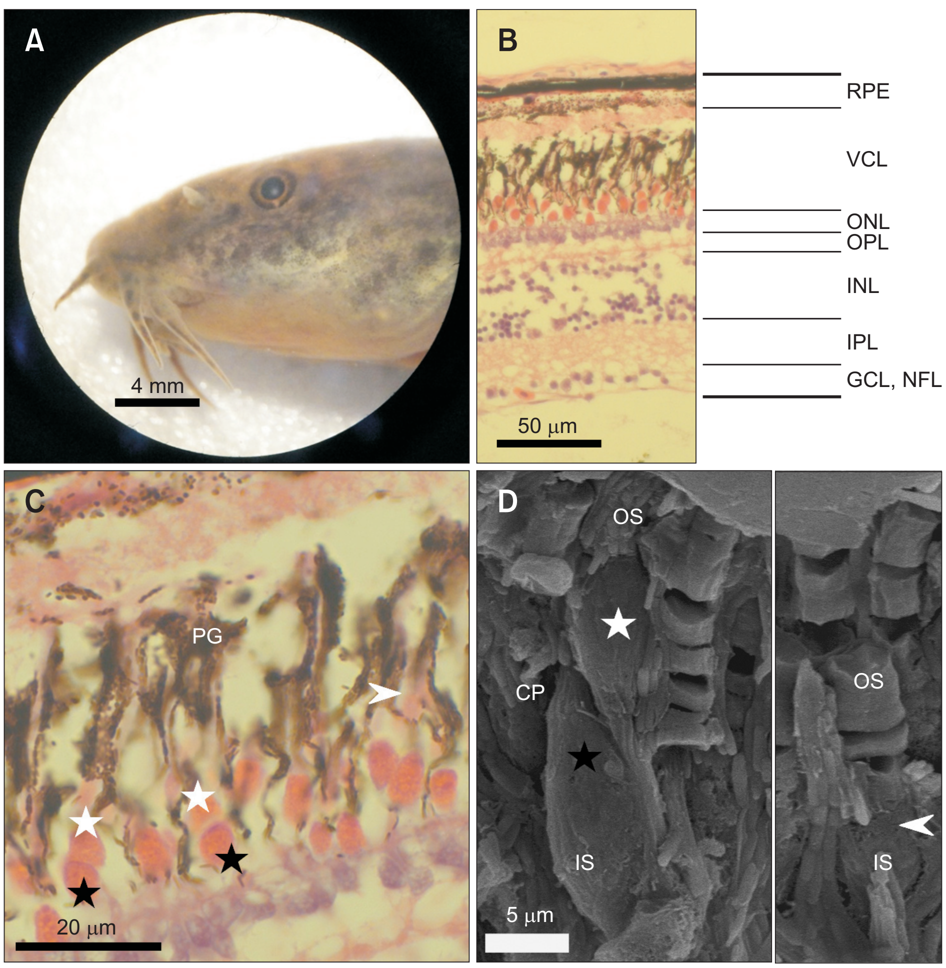

Fig. 1. External eye morphology and gross sections of the retina in a Harris hematoxylin and counter-stained with eosin used by light microscope and scanning electron microscope of *Misgurnus anguillicaudatus.* (A) Left eye side view. (B, C) Longitudinal sections of the retina. (D) Scanning electron micrograph. RPE, retina pigment epithelium; VCL, visual cell layer; ONL, outer nuclear; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, nerve fiver layer; PG, pigment granules; OS, outer segment; CP, calyceal process; IS, inner segment. Black asterisks, short cones; white asterisks, big cones; arrowheads, rod cells.

pigment epithelial layer, a visual cell layer, an outer nuclear layer, an outer plexiform layer, an inner nuclear layer, an inner plexiform layer, and a ganglion cell layer (Fig. 1B). In particular, the retinal pigment epithelium is the pigmented cell layer of pigment grains, melanin granules, extending toward photoreceptor outer segments beneath a choroid layer filled with blood vessels. The visual cell layer consists of a dense packing of both double cone cells and rods (Fig. 1C and 2A).

Structure of the Rod Cell and Cone Cell

The large rods typically have a single layer with a long and rod-shaped outer segment and a shorter inner segment. The outer segment is acidophilic, staining with hematoxylin, and surrounded by plenty number of pigment epithelial cells, whereas the inner segment is basophilic or eosinophilic (Fig. 1C). As the rods is thinner and longer cell than cone cell, it is very difficult to find it through LM. By the semi-thin sections, however, the existence of the rod cells become clear (Fig. 2A). The outer segment is positive with toluidine blue and the ellipsoid is also somewhat positive, whereas the myoid region is negative. They reach up to a mean of 51.4±2.6 μm in length and 3.5±0.5 μm in diameter.

The double cones are a symmetrical shape and unequal in length, with the same staining appearance. The double cones consist of two elements, big and small cones: a big cone with 24.0±2.1 μm in length and 4.9±0.4 μm in diameter (n=30) and a small cone with 15.9±1.1 μm in length and 5.2±0.3 μm in diameter (n=30) (Fig. 1C and D). The double cone cells are variable in size, and they are generally shorter than the rod cells and have a thicker diameter. In contrast to the rod cells, the outer segment is short and conical, whereas the inner segment is large and bulbous. The cell extensions reach the outer plexiform layer. Most of big elements are eosinophilic, and most small elements are hematoxylinophilic (Fig. 1C). In the semi-thin sections stained with toluidine blue, the outer segments are weakly stained but the inner segments are strong positive (Fig. 2A). In tangential section, the double cones show a mosaic row pattern in which the contact zones between the partners of the double cones are parallel (Fig. 2B-D). The double cones do not have a homogeneous distribution because they are comprised big and small cones.

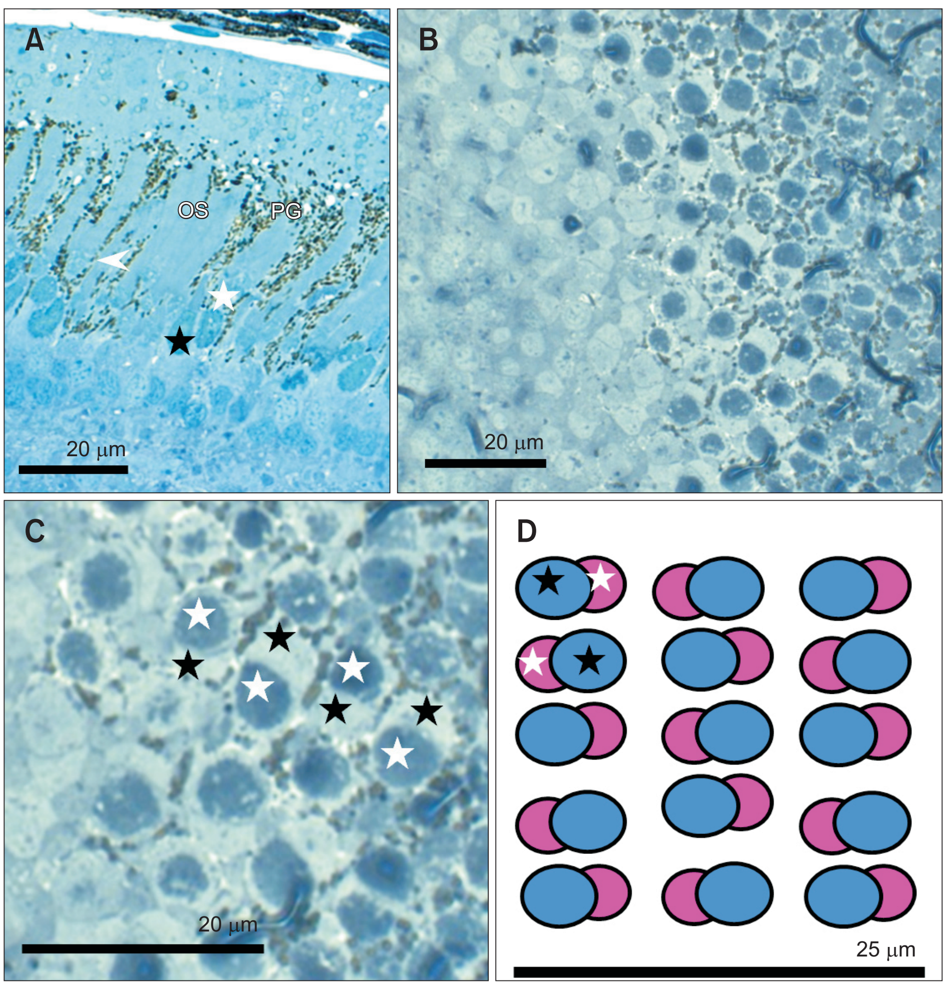

Fig. 2. Visual cell layer by semi-thin sections in the *Misgurnus anguillicaudatus* retina. (A) Longitudinal sections of the visual cell layer. (B, C) Transverse sections showing a mosaic model in the visual cells by light microscopy. (D) Diagram based on the left micrograph. OS, outer segment; PG, pigment granules. Black asterisks, short cones; white asterisks, big cones; arrowheads, rod cells.

The double cones are rotated in relation to the long axis, thus forming parallel lines. The rod cells are spaced at equal distances, forming rows parallel to rows of double cones. With SEM, it is apparent that the outer segments are linked to the inner segment by so-called "calyceal process" (Fig. 1D), unlike rod cells.

DISCUSSION

The family Cobitidae is known over 260 species in the world, but the study about visual cells is restricted to three Indian fishes, *Nemacheilus beavani*, *Nemacheilus devdevi*, and *Balitora brucei*. In *N. beavani* and *N. devdevi* of the mountain-stream teleosts have few rods, short and long single cones and unequal double cones showing a square mosaic pattern, whereas other hill-stream loach, *B. brucei*, has rods, double cones and single cones (short as well as long types), not showing a defined cone mosaic pattern unlike the above two cobitid fishes (Nag & Bhattacharjee, 2002). These structures may reflect any ecological backgrounds: the bottom-dwelling insectivores and better adaptation for clear water of the mountain streams having swift currents. It is also said that the row patterns of the cones, on the other hand, are present in the predominantly insectivorous forms (*Danio aequipinnatus* and *Barilius* spp.) that prefer to swim near the surface.

In teleost fishes, the cone pattern is an arrangement of four equal, double cones surrounding a single cone (Engström, 1963; van der Meer, 1992). This pattern may contain either central or additional single cones or both; however, in some fish, the single cone may be absent (O'Connell, 1963; Anctil, 1969; Boehlert, 1979). In cone pattern of double cone, it has largely two types, row and square patterns of double cone: the former is especially suitable for vision in dark homochromatic environments, while the latter has a high adaptive capacity to varying spectral distributions in luminous environments (Kunz, 1980; van der Meer, 1992).

Unlike the above three loaches, however, the Korean pond loach *M. anguillicaduatus* forms a row mosaic pattern in which the partners of double cones are linearly oriented with few large rods. In a double cone or twin cone, the two members are unequal such that one cone may be longer than the other. Such double cones are thought to be formed by the fusion of single cones (Engström, 1963; Wheeler, 1982) and primarily sensitive to medium and long wavelengths, whereas single cones are primarily sensitive to the shorter blue wavelengths (Bowmaker, 1995; van der Meer & Bowmaker, 1995). The rod cells are varied in shape and disposition with long stout outer segments or short and thin one (Nicol, 1989; Wagner, 1990).

M. anguillicaduatus is a diurnal-bottom dweller that inhabits muddy or silt strata of shallow and stagnant water such as pond, reservoirs and rice-fields and mainly feed on algae rather than on aquatic insects (Kim & Park, 2002). Regarding the cone mosaic pattern, there are contradictory hypotheses: 1) it is important for detection in luminous environments (Kunz, 1980), 2) a strategy adapted well for the hunting activity of predatory fish (Rossetto et al., 1992), and 3) a close link to ecology rather than food habits or photic environments (Nag & Bhattacharjee, 2002). In our study, therefore, the structure of the visual cells of *M. anguillicaduatus*, is more likely to have a close relation with its ecological factors.

CONCLUSIONS

Visual cells in the retina of *M. anguillicaudatus* has a row mosaic pattern in which the partners of double cones consisting of big and small ones are linearly oriented with large rods. This species, a diurnal-bottom dweller, inhabits slow and shallow water of rivers and streams or calm habitats such as swamps, reservoirs and paddy fields, with substrates composed of mud or slit. Consequently, the pattern of the visual cell appears to have a possibility that may reflect microhabitat of this species.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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